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IMMUNE REACTIONS IN SCARLET FEVER*

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Some time ago[1] we reported the results of anaerobic cultures in scarlet fever. Cultures were made from the throat, blood, lymph glands, kidneys, and urine. This work indicated that during the acute stage of scarlatina a variety of organisms enter the blood and tissues and are excreted, in part at least, through the kidneys. It was not clear from our animal experiments whether any of these organisms caused scarlet fever or whether all existed only as accidental or secondary invaders. Some of the animals died with suspicious rashes, but nothing was obtained which could be called an experimental scarlet fever.

Before studying further the significance of the organisms obtained in cultures, it seemed desirable to test the various fluids from which they were grown in order to determine whether or not a virus or antigen was present that perchance is ultramicroscopic, or which fails to grow with the cultural methods in use. If such could be demonstrated, the secondary importance of the organisms described would seem to be clear.

In testing for the presence of such a virus or antigen, the immune reactions were used. In addition, the blood serum was tested as to toxicity for animals and for the presence of living virus by means of potassium tellurid. The agglutination test had been used with some of the organisms isolated and had proved so unsatisfactory that in the work reported here, the complement-fixation and cutaneous reactions only were used. The antigenic properties of the blood, the throat and mouth secretion, extracts of spleen, and extracts of lymph glands were examined. The results follow.

BLOOD

Complement-Deviation Test.—Antigens in the body fluids have been found by means of complement-fixation in a number of instances. For example, tuberculous antigens were demonstrated in urine and pleuritis

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* Received for publication February 5, 1916.

[1] Dick, G. F. and G. R.: Jour. Infect. Dis., 1914, 15, p. 85.

exudates with known tuberculous antiserum.[2] We have tested antigenic properties of 6 sera by complement-fixation.

These sera were obtained from scarlatinal patients at the height of intoxication as indicated by the temperature, rash, etc. Antisera for these tests were obtained from 8 convalescent scarlet-fever patients at periods varying from the end of the 1st to the end of the 6th week. Sheep erythrocytes and antisheep rabbit serum with fresh guinea-pig serum as complement were used. For each test the antisheep rabbit serum was titrated with a constant amount of guinea-pig serum, and 0.05 c.c. of complement and 2 units of rabbit amboceptor used. Increased power of hemolysis (both amboceptor and complement) in scarlatinal serum has been described by a number of observers, notably Moro.[3] In this work, in order to guard against interference with fixation by such hemolysins in the serum used as antigen, each serum was divided into 3 parts, each one of which was used for a complete reaction. In the 1st series, the active serum was used as antigen; in the 2nd series, serum heated at 56 C. for one-half hour; in the 3rd series inactivated serum, from which the sheep amboceptor had been removed through absorption by sheep cells. In order to correct the increased power of hemolysis for sheep cells in the convalescent serum, the hemolytic power of the serum was titrated, and, if hemolysin was present, it was inactivated. If the inactivated serum showed an excess of amboceptor, the amboceptor was extracted by excessive amounts of sheep cells. The amount of convalescent antiserum used was 0.1 c.c. in all the tests. The quantities of antigen, that is, serum of early scarlet fever, used were: 0.025 c.c., 0.05 c.c., 0.1 c.c., 0.15 c.c., 0.2 c.c., 0.3 c.c., and 0.5 c.c. The amount of sheep cells used was 0.5 c.c. of a 5% suspension. For each test a control antiserum was used which had been obtained from convalescent diphtheria patients who gave no history of scarlet fever. All the sera used proved negative in Wassermann tests with acetone-insoluble beef-heart-extract antigen.

In all the various ways in which the tests were carried out there was no evidence of complement-fixation. The diphtheria controls were also negative. Kappel[4] obtained negative results in testing the sera of 20 patients in the acute stage of scarlet fever for the presence of antigen by means of complement-fixation. He used only inactivated serum without correction for increased natural amboceptor, so that the test was probably not so delicate as those described here. Results of both methods, however, indicate that complement-fixation fails to demonstrate any specific antigen in the serum of scarlatinal patients as early as the 2nd day of the disease.

Toxicity of the Serum of Scarlatinal Patients.—That there is a difference between normal serum and the serum of patients sick with scarlet fever was shown by Hamilton[5] on Peramœcium, Syrensky,[6] and more recently also by Bankowski and Szymanowski.[7]

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[2] Delse and Parap: Compt. rend. Soc. de biol., 1911, 71, p. 228.

[3] Wien. med. Wchnschr., 1908, 8, pp. 3, 39, 159.

[4] Wien. klin. Wchnschr., 1911, 24, p. 1295.

[5] Jour. Infect. Dis., 1903, 1, p. 211.

[6] Ztschr. f. Immunitätsf., 1914, 20, p. 543.

[7] Ibid., 1913, 16, p. 330.

The last two observers found the serum of scarlatina patients fatal for guinea-pigs in doses of from 0.13 c.c. to 0.4 c.c. per 100 grams' weight of guinea-pig. The serum of normal individuals was fatal for guinea-pigs in doses of from 0.5 to 0.6 c.c. per 100 grams' weight of guinea-pig. Syrensky allowed the blood that was to be tested for toxicity to stand for from 6 to 12 hours before injection. Bankowski and Szymanowski do not mention this interval. Mita and Ito[8] show that after one-half hour the toxicity of foreign serum for guinea-pigs decreases; so that after from 6 to 24 hours it becomes only a third as toxic as when freshly drawn.

In the following experiments blood was collected in centrifuge tubes and the serum separated and injected into guinea-pigs intravenously within one-half hour after withdrawal from the patient. Sera from 2 mildly sick scarlet fever patients were compared with sera from 2 convalescents. The minimal lethal doses of the former were 0.25 c.c. and 0.39 c.c. per 100 grams' weight of guinea-pig. Those of the latter were 0.43 c.c. and 0.5 c.c. per 100 grams.

These results, while less striking than those noted by other observers, indicate that the blood of scarlet-fever patients is more toxic during the time the patient is acutely sick than it is during convalescence. Indeed, from the reports of other observers, the toxicity of acute scarlet-fever serum, unlike normal serum, tends toward an increase on standing rather than a decrease.

Potassium-Tellurid Test.—It has long been known that tellurium compounds dissolved in growing cultures of micro-organisms are broken up and the tellurium absorbed by the micro-organisms. King and Davis[9] have shown that for many bacteria potassium tellurid dissolved in the media in the proportion of 1 : 10,000 to 1 : 300,000, acts as an efficient indicator of microbic growth. The bacteria absorb the tellurium and are precipitated at the bottom of the culture tube in the form of a black button. Experiments were undertaken to see if any virus could be demonstrated in the serum of scarlet fever which could not be detected by the cultural methods used in the work previously reported.

For this purpose tubes of about 10 c.c. of ascites broth (1 :10) containing potassium tellurid were inoculated with 1 c.c. of scarlet-fever serum obtained from acutely sick patients. Six tubes were made for each case of scarlet fever, 3 aerobic and 3 anaerobic, each set containing tellurid in dilutions of 1 :20,000, 1 :40,000, and 1 :100,000. Six simular tubes without serum were used as negative controls in each test. Six positive controls, aerobic and anaerobic, were made by inoculating ascites-broth tellurid dilutions with small gram-negative diphtheroids from the urine of a scarlet-fever patient.

In the sera from 6 cases of scarlet fever there was no precipitation of tellurium more marked than in the negative control tests, which became affected to some extent after about one week's incubation. The positive controls showed marked precipitation in every instance. The test was not so satisfactory for the purpose as might have been hoped from the work of King and Davis,[8] as the differences between positive and negative results, while clear enough for such rapidly growing organisms as they used in their work, were not so clear after 10 days' incubation. Another disadvantage of the test is that it is not adapted to those methods of culure which gave positive results in the work reported previously. The experiments with tellurid are of value only in showing that there probably are no rapidly multiplying organisms present in scarlet-fever serum which grow in ascites tellurid broth.

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[8] Ztschr. f. Immunitätsf., 1913, 17, p. 586.

[9] Jour. Pub. Health, 1914, 4, p. 917.

SPLEEN

Extract of spleen for antigen was prepared as follows: The spleen of a child who had died on the 2nd day of scarlet fever—the blood culture during life having been free from streptococci—was ground in a mortar with 0.5% phenol in 0.85% salt solution and the mixture allowed to stand in the ice-box over night. In the morning the supernatant fluid was removed and used as antigen.

Complement-Fixation.—Following a method of fixation similar to that described for the tests with blood serum, tests were made with the sera of 6 cases of scarlet fever at various stages, from the 2nd day to the 3rd week of the disease.

The antigen was used in amounts of 0.025 c.c., 0.05 c.c., 0.1 c.c., 0.2 c.c., 0.3 c.c. In 0.3 c.c. the antigen alone inhibited hemolysis, hence no higher strengths were used. The serum was used in the constant quantity of 0.1 c.c. As controls, the antiserum used was tested with acetone-insoluble heart extract, and the antigens with sera of diphtheria patients as antisera. No serum tested showed complement-fixation. Indeed, in some instances the tubes containing scarlet-fever serum and antigen hemolyzed in strengths of antigen which inhibited both alone and with diphtheria serum.

Cutaneous Tests.—With the same spleen which was used for complement-fixation, an extract was made with 0.085% salt solution and evaporated at 60 C. to 1/10 its volume; enough phenol then was added to make 0.5%. Before use of the antigen sterility was determined by cultures. In 4 convalescent scarlet-fever patients and 4 diphtheria patients tests made with this antigen similar to the tuberculin cutaneous tests gave uniformly negative results.

LYMPH GLANDS

Lymph glands were collected from various parts of the same body from which the spleen just described was obtained. An antigen was made for complement-fixation by the same method, and fixation tests carried on with the same sera as antisera, that were used with the spleen tests.

The amounts of antigen used were 0.025 c.c., 0.05 c.c., 0.1 c.c., 0.2 c.c., 0.3 c.c., and 0.4 c.c. In the last two quantities the antigen alone gave a partial inhibition. The sera from the scarlet-fever patients were used in 3 ways—active, inactive, and after removal of natural amboceptor by means of washed sheep cells. Controls were used as in the case of the extract of the spleen.

The results of these tests were uniformly negative. Cutaneous tests with lymph-gland extract made in a way similar to those with the spleen extract were negative in 3 cases of scarlet fever and in 3 controls.

The results of complement-fixation tests with organ extracts are in harmony with the results obtained by some observers and at variance with those obtained by others. Following the introduction of the Wassermann test with extract of syphilitic liver as antigen, Haendel and Schultz[10] substituted extract of scarlatinal liver as antigen and obtained 24 positive reactions in 31 cases of scarlatina tested. Uppenheimer[11] obtained negative results with extract of scarlatinal liver as antigen. Sommerfeld,[12] using syphilitic-liver extracts and scarlatinal-liver extracts as antigen, found that some scarlatinal sera fixed complement with both kinds of antigen. There was no relation to the severity of the disease. Scarlatinal serum which fixed with one syphilitic-liver extract failed to do so with other syphilitic-liver extracts, tho both extracts reacted equally well with the sera of syphilitic patients. Hecht, Pelissier, and Wileno[13] obtained negative results in 119 cases in which fixation was tried with alcoholic extracts of scarlatinal liver as antigen. Kolmer[14] tested 250 cases for complement-fixation with alcoholic extracts of both syphilitic and scarlatinal livers and obtained positive results in 2% with syphilitic liver and in 2.4% with scarlatinal liver. In none of the positive cases could syphilis be excluded. He concludes that complement-fixation with scarlatinal liver as antigen and scarlatinal serum as antibody gives negative results. Kappel,[4] testing as antigen the serum, extract of desquamated scales, and watery and alcoholic extract of tonsils of scarlatina, obtained negative results. The Koesslers[15] used extracts of liver, kidney, and lymph glands as antigen.

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[10] Ztschr. f. Immunitätsf., 1908, 1, p. 91.

[11] München. med. Wchnschr., 1909, 6, p. 2471.

[12] Arch. f. Kinderh., 1909, 50, p. 38.

[13] Ztschr. f. Immunitätsf., 1909, 2, p. 356.

[14] Jour. Exper. Med., 1911, 14, p. 235.

[15] Jour. Infect. Dis., 1911, 9, p. 366.

With liver extracts 32% of the tests were positive, with kidney extracts 57%, and with lymph-gland extracts 68%. They consider that they have demonstrated the specific virus of scarlet fever as being present especially in the lymphatic glands. Isabolinsky and Legeika[16] used both watery and alcoholic extracts of scarlatinal liver, spleen, and lymph glands. Unlike the Koesslers, they obtained positive reaction in both scarlatinal and control sera and conclude that the fixation obtained is not specific.

TABLE 1

THE PERCENTAGE OF CASES OF SCARLET FEVER GIVING POSITIVE WASSERMANN TESTS ACCORDING TO VARIOUS OBSERVERS

Observer	Kind of Antigen Employed	Percentage Positive
Much and Eichelberg[1]	Watery extract of syphilitic liver	40
Teissier and Bernard[2]	Watery extract of syphilitic liver	84
Haendel and Schultz[3]	Watery extract of syphilitic liver	13
Jochmann and Toepfer[4]	Watery extract of syphilitic liver	3
Jacobovics[5]	Not stated	33
Zeissler[6]	Alcoholic extract of human heart	4.8
Bruck and Cohn[7]	Alcoholic extract of syphilitic liver	2.8
Boas and Hange[8]	Alcoholic extract of human heart	1.6
Hecht, Lateiner and Wilenko[9]	Alcoholic extract of guinea-pig heart	1
Meier[10]	Watery extract of syphilitic liver	0
Schleissner[11]	Alcoholic extract of guinea-pig heart	0
Seligman and Klopstock[12]	Alcoholic extract of human heart:	
	1st series	0
	2nd series deteriorated antigen	100
Halberstaedter, Müller, and Reiche[13]	Alcoholic extract of syphilitic liver:	
	One antigen	50
	All other antigens	0
*Kolmer[14]	Alcoholic extract of syphilitic liver	2
Gross and Folk[15]	Alcoholic extract of human heart	0
Hoehne[16]	Alcoholic extract of syphilitic liver	0
Sommerfeld[17]		0
Noguchi[18]	Acetone-insoluble extract of beef heart	0
*Author's observations	Acetone-insoluble extract of beef heart controlled in 6 cases by cholesterinized alcoholic extract of human heart	1
Fua and Koch[19]		0

* Syphilis not excluded.
[1] Med. Klin., 1908, 4, p. 671.
[2] Compt. rend. Soc. de biol., 1910, 68, p. 272.
[3] Ztschr. f. Immunitätsf., 1908-9, 1, p. 91.
[4] München. med. Wchnschr., 1908, 4, p. 1690.
[5] Jahrb. f. Kinderh., 1914, 29, p. 215.
[6] Berl. klin. Wchnschr., 1908, 14, p. 1887.
[7] Ibid., p. 2268.
[8] Ibid., p. 1566.
[9] Ztschr. f. Immunitätsf., 1909, 2, p. 356.
[10] Med. Klin., 1908, 4, p. 1383.
[11] Wien. klin. Wchnschr., 1908, 21, p. 1375.
[12] Berl. klin. Wchnschr., 1908, 45, p. 1710.
[13] Ibid., p. 1917.
[14] Jour. Exper. Med., 1911, 14, p. 235.
[15] Wien. klin. Wchnschr., 1908, 21, p. 1522.
[16] Berl. klin. Wchnschr., 1908, 14, p. 1717.
[17] Arch. f. Kinderh., 1909, 1, p. 38.
[18] Serum Diagnosis of Syphilis, 1910.
[19] Wien. klin. Wchnschr., 1909, 22, p. 522.

In interpreting these varying results much valuable information may be obtained by a review of the Wassermann test in scarlet fever. Much and Eichelberg,[17] using watery extract of syphilitic liver as antigen, obtained positive results in 40% of scarlet-fever cases, and they concluded that the Wassermann reaction was not specific for syphilis.

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[16] Centralbl. f. Bacteriol., I, O., 1913, 71, p. 520.

[17] Med. Klin., 1908, 6, p. 671.

Following this work, which unfortunately has given rise to the idea that the Wassermann test is commonly positive in scarlet fever, a few other reports appeared giving positive results. These reports are summarized in Table 1. It will be seen that the watery extracts of organs gave the highest percentage of positive tests as well as the greatest variation in the hands of different observers. The alcoholic and acetone-insoluble extracts, except when deteriorated, give practically uniformly negative results in scarlet-fever patients in whom syphilis could be excluded, both according to others' results and according to our own results in 100 cases.

The work of Seligman and Klopstock and Halberstaedter illustrates what variation in results can be obtained with a change in the antigen employed. The watery extracts used as antigen gave such a high percentage of positive results with normal and other sera that they have been abandoned as antigens for the Wassermann test for syphilis. From the foregoing considerations it is plain that positive results in fixation tests in which watery extracts of organs serve as antigen, must be interpreted with great reserve. Recently Schultz[18] used washed scarlatinal lymph glands as antigens for Abderhalden reactions in scarlet fever. He obtained positive results with the sera of scarlatina patients, but he also obtained positive results when he used normal lymph glands as antigen. He concludes that the reaction is not connected with any specific virus for scarlet fever.

THROAT AND MOUTH SECRETIONS

Tho not proved, it is commonly assumed that the angina associated with scarlet fever is the source of a general intoxication due at least to secondary infection if not to the specific virus of the disease. Mucus from the throat and mouth of cases with active angina was collected and used as antigen for complement-fixation with a method such as described in the foregoing tests.

The mucus from cases with active angina was suspended in salt solution and by preliminary titration a concentration obtained which made convenient quantities. The following quantities were used: 0.01 c.c., 0.02 c.c., 0.03 c.c., 0.05 c.c., 0.1 c.c., 0.15 c.c., 0.2 c.c., 0.3 c.c., and 0.5 c.c. The mucus was used fresh in all but one instance; here it was used both fresh and heated to 60 C. for 1 hour. The serum was employed in constant quantity, 0.1 c.c., and inactivated except in one test, in which it was used in both active and inactive form. For each test a control with serum from diphtheria patients was made. As in all the experiments, Wassermann tests were made with serum from scarlet fever. In one case no inhibition was obtained by means of antigen alone in any of the quantities used. In the other tests the antigen produced partial inhibition in the highest quantities.

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[18] München. med. Wchnschr., 1913, 60, p. 2510.

The results of the tests were as follows: In sera from 4 cases of scarlet fever in various stages of convalescence, each used with a different antigen, there was no evidence of complement-fixation. As a rule the diphtheria serum inhibited hemolysis with lower strengths of antigen than did the scarlatinal serum. In a 5th case there was a slight fixation of complement with 0.3 c.c. of antigen. There was no fixation of complement at all in the control up to 0.5 c.c. The same antigen failed to fix complement with the serum of a 6th case of scarlet fever.

SUMMARY

Complement-deviation and cutaneous tests failed to demonstrate any specific scarlatinal virus or antigen in the blood serum or in extracts of spleen or lymph glands.

Fixation tests with throat mucus as antigen gave in one instance a weak fixation of complement with the serum of a convalescent scarlatinal patient.

On the basis that insufficient concentration of antigen explains the negative results, it would appear desirable to study further the cultures obtainable from scarlet fever, particularly as to their immune reactions.

The toxicity of human blood serum for guinea-pigs is increased during the acute stage of scarlatina.

Positive Wassermann reactions in scarlet fever obtained with the acetone-insoluble portion of alcoholic heart extract are strongly suggestive of syphilis.